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Self-Immolative Comb-Polymers: Multiple-Release of Side-Reporters by a Single Stimulus Event

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Chemists have developed several sophisticated strategies in order to achieve amplification of molecular signals.[1–3] Self-immolative molecular systems are molecules that disintegrate into their building blocks through domino-like reactions upon a single triggering stimulus. We and others have previously reported on self-immolative dendritic molecules that amplify a single triggering reaction at the focal point into release of multiple peripheral reporter groups.[4–6] However, the number of groups that can be incorporated on a dendrimer periphery is limited due to steric hindrance and higher dendrimer generation requires additional synthetic steps.[7] In order to overcome such disadvantages, we recently introduced a novel kind of linear "smart" polymer:[8,9] These polymers respond to external stimuli by undergoing head-to-tail disassembly through a self-immolative fragmentation once the head-trigger is activated.[10] Disassembly of this type of polymer to release monomers with strong fluorescence signal was demonstrated in context of a molecular sensor with large signal-to-noise ratio.[10] Here, we report synthesis of the next generation of self-immolative polymers, which represent a significant step forward. Appropriate incorporation of side reporters (R) on each monomer and a trigger at the head-monomer generated a self-immolative comb-polymer with releasable side reporters. (Figure 1) Removal of the trigger initiated polymer disassembly along the backbone into its building block, followed by spontaneous release of the reporters from each monomer.

Self-immolative comb-polymers can be designed based on building blocks such as **1** (Scheme 1). This molecule can undergo double-elimination reactions to release its two reporters. The disassembly is initiated by cleavage of the trigger to unmask amine **2**. The latter spontaneously undergoes 1,6-elimination to release a reporter (R^1) and azaquinone methide **3**. This reactive intermediate is rapidly trapped by an available nucleophile to form amine **4**. Additional 1,6-elimination, this time at the vinylogous *ortho*-benzyl position, releases a second reporter unit (R^2) and compound **5**, which subsequently reacts with additional nucleophile to form amine **6**. The azaquinone–methide rearrangement can take place either through an *ortho*-benzyl[11] or through a vinylogous *ortho*-benzyl position. Our preference to use for a vinylogous *ortho*-benzyl substituent is based on the synthetic strategy presented in the Supporting Information.

We designed self-immolative comb-polymer **7** based on **1** (Scheme 2). The polymeric backbone is constructed of polyurethane. 4-Nitroaniline was used as a reporter molecule and 4-hydroxy-2-butanone, which is designed for removal through a β-elimination reaction by piperidine, was applied as a model trigger.[10] Removal of the trigger of the polymer head should initiate the polymer disassembly along its backbone and the release of multiple reporter units.

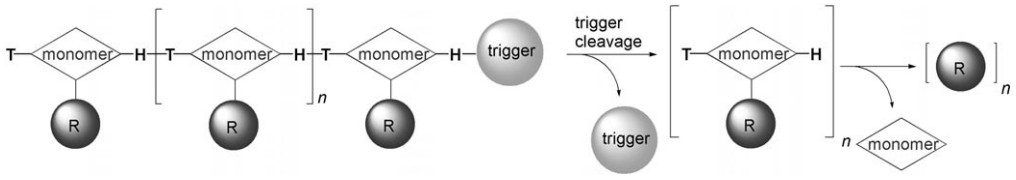

Figure 1. Illustration of the disassembly of a self-immolative comb-polymer.

In order to study the disassembly mechanism presented in Scheme 1, we synthesized monomeric system **8** (Scheme 3). This molecule has two different reporters (1-naphthylamine

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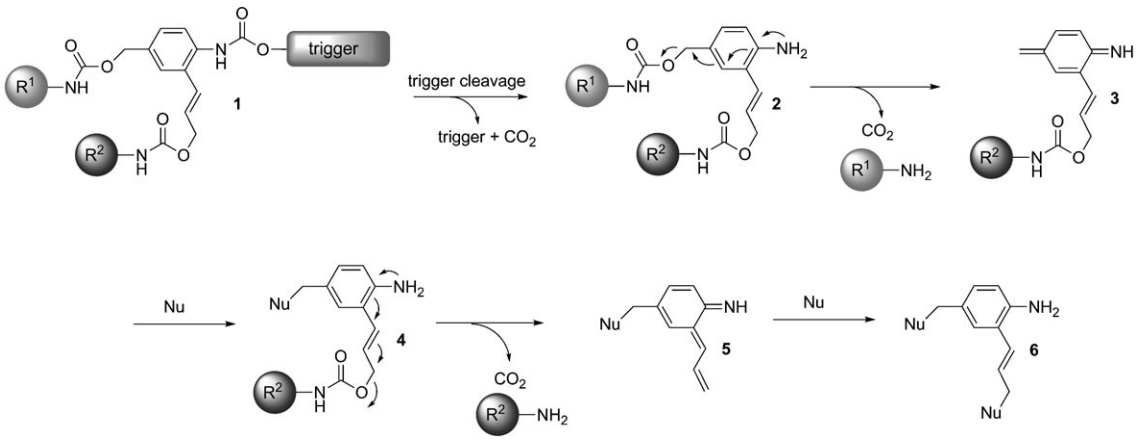
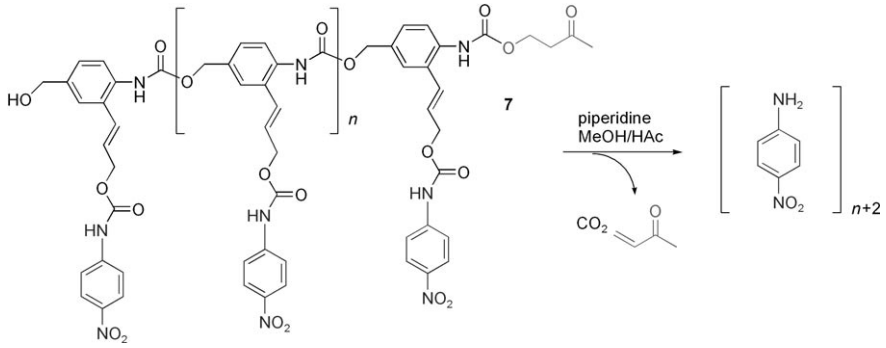


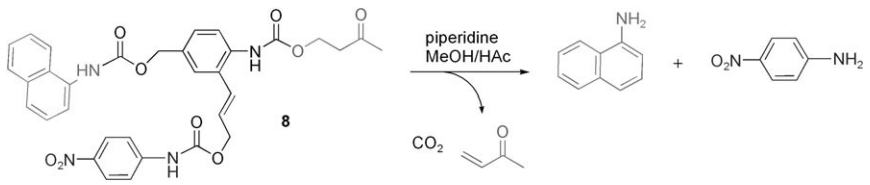
Scheme 1. Design and disassembly mechanism of a self-immolative unit of a comb-polymer.



Scheme 2. Piperidine initiates disassembly of self-immolative polymer **7** to release multiple copies of the 4-nitroaniline reporter units.

and 4-nitroaniline) and a 4-hydroxy-2-butanone trigger. Compound **8** was incubated with piperidine in methanol to activate the trigger and disassembly to release 1-naphthylamine and 4-nitroaniline was monitored by RP-HPLC.

The 1,6-elimination to release 1-naphthylamine occurred rapidly (within less than 1 h), whereas the second elimination through the vinylogous *ortho*-benzyl position prolonged over 20 h to release the second reporter 4-nitroaniline (Figure 2). Importantly, the proposed double elimination of **2** to release two reporters occurred as expected. In a control reaction with an identical compound and a trigger that is not cleaved by piperidine (4-methylbenzylalcohol), almost no release of the reporters was observed (data not shown). These results support the disassembly pathway of **1** suggested above (Scheme 3).



Scheme 3. Piperidine initiates disassembly of self-immolative compound **8** to release two reporter units: 1-naphthylamine and 4-nitroaniline.

Next, we sought to study the disassembly of a self-immolative comb polymer. Polymer **7** was synthesized by polymerization of monomer **9** according to a procedure we recently developed (Scheme 4).[10] The polymer head was capped with the trigger 4-hydroxy-2-butanone to generate a polymeric compound with an average of 11 building blocks (determined by NMR). The polymer was incubated in MeOH/DMSO solution with or without piperidine/acetic acid and the release of 4-nitroaniline was monitored by RP-HPLC (Figure 3).

The concentration of free 4-nitroaniline gradually increased over 48 h when piperidine was present in the polymer solution. In the absence of piperidine, almost no free 4-nitroaniline was observed (about 10% after 50 h). As a control experiment we used similar polymer with a 4-methylbenzylalcohol capping group instead of 4-hydroxy-2-butanone (see polymer **7a** in the Supporting Information). Since this capping group does not undergo β-elimination in the presence of piperidine, almost no release of 4-nitroaniline was observed in this control reaction.

The disassembly of polymer **7** was evaluated in organic solvents and was found to be relatively slow. Previous results showed a faster elimination rate for azaquinone–methide generation under aqueous conditions.[12] In addition, we sought to evaluate the potential of our polymers as drug delivery systems. Therefore, we developed a water-soluble version of a self-immolative comb-polymer. Monomer **10** was designed from two aniline subunits. The first contains a

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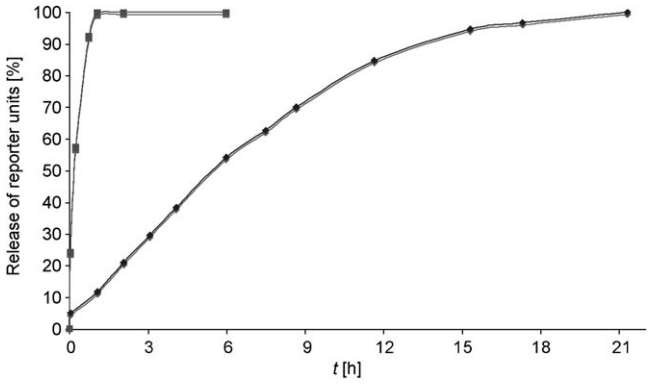
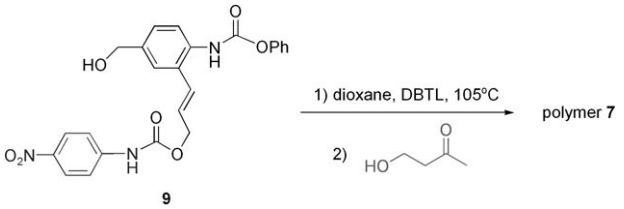


Figure 2. Release of 1-naphthylamine (■) and 4-nitroaniline (◆) from compound **9** upon exposure to piperidine/acetic acid.



Scheme 4. Synthesis of self-immolative comb-polymer **7**.

releasable side-reporter and the second has a *tert*-butylacrylate substituent that can be deprotected with TFA after the polymerization to afford polymer **11**. Polymer **11** has an ionized carboxyl functional group on every other aromatic

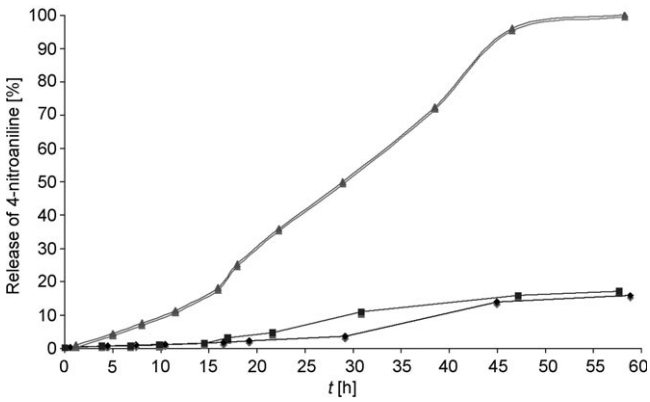
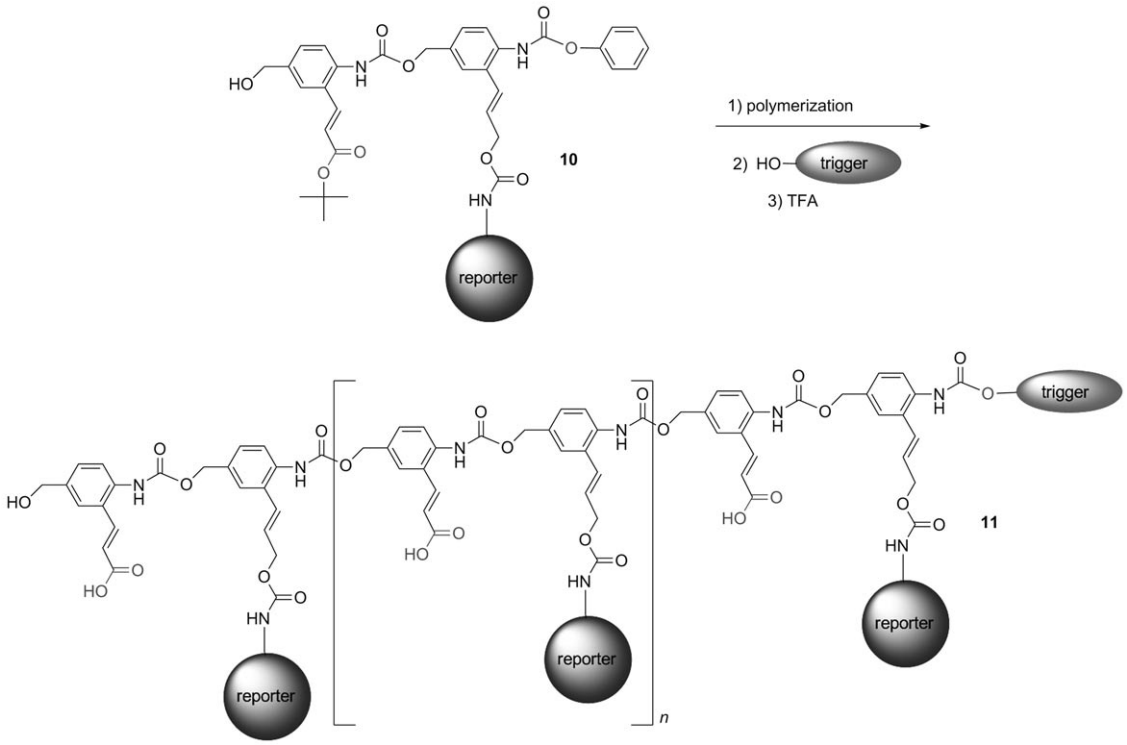


Figure 3. Disassembly (▲) of self-immolative comb-polymer **7** upon treatment with piperidine/AcOH to release 4-nitroaniline. Polymer **7** without treatment of piperidine/AcOH (◆). Polymer **7a** upon treatment with piperidine/AcOH (■).

building block and thus is expected to be soluble under physiological conditions.

Self-immolative comb-polymer **12** was prepared according to Scheme 5 from a monomer that carries 4-nitroaniline as a reporter unit (a polymer with an average of 7 building blocks was obtained). The polymer head was capped with a trigger designed for removal by penicillin-G amidase (PGA).[13] The enzyme initiates the polymer disassembly to release its reporter units by a single cleavage of the phenylacetamide moiety, followed by 1,6-elimination and decarboxylation reactions (Scheme 6).



Scheme 5. Synthesis of a water-soluble self-immolative comb-polymer with a trigger.

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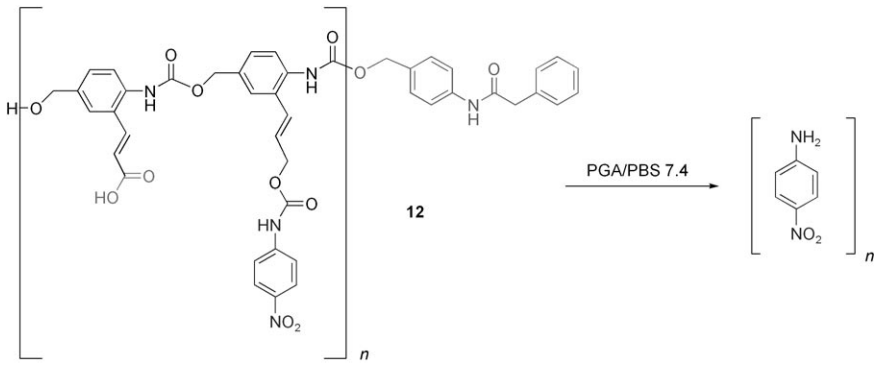

Scheme 6. PGA-catalyzed the disassembly of self-immolative comb-polymer **12** to release 4-nitroaniline.

The polymer was incubated in phosphate buffer saline (PBS), pH 7.4, in the presence and in the absence of PGA and the release of 4-nitroanilne was monitored by RP-HPLC (Figure 4). The polymer disassembly indeed occurred faster under aqueous conditions. The release of 4-nitroanilne was completed within 6 h (vs 48 h in organic solvent) when PGA was added to the polymer solution. About 20% release was observed in the background reaction as a result of spontaneous hydrolysis of the relatively labile 4-nitroanilne carbamate derivative. Additional example for activation and disassembly of a self-immolative comb-polymer with BSA (bovine serum albumin) is described in the Supporting Information (polymer **25**).

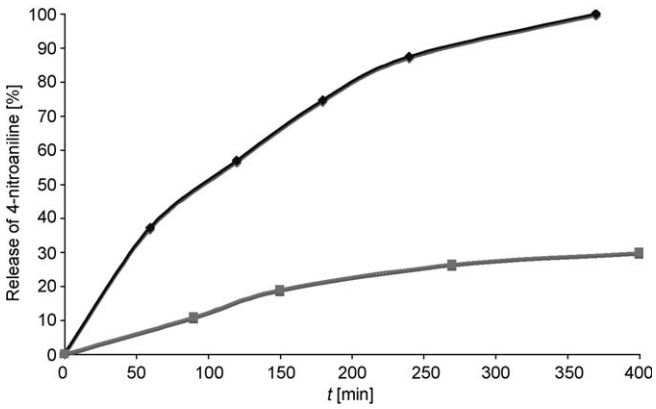

Figure 4. Disassembly (◆) of self-immolative comb-polymer **12** upon addition of PGA to release 4-nitroaniline. Control reaction: polymer **12** in PBS, pH 7.4 (■).

The protracted release of the side reporters can be applicable in drug delivery systems that require a slow-release procedure. Incorporation of a drug molecule instead of the reporter in the polymeric molecule will afford a comb-polymer that can release multiple drug units upon a single cleavage event. Polymeric molecules with molecular weight over 20 kD are known to accumulate selectively at tumor sites due to the enhanced permeability and retention (EPR) effect.[14] This effect occurs due to the difference between the vasculature physiology of solid tumors and normal tissues. The growth of the tumor creates a constant need for the continuous supply of new blood vessels. This process, termed angiogenesis, often results in the construction of vessels with leaky walls, which allow enhanced permeability of macromolecules within the tumor. In addition, poor lymphatic drainage at the tumor site promotes accumulation of large molecules. Self-immolative comb-polymers loaded with a chemotherapeutic drug and a trigger that is activated by a specific enzyme overexpressed in tumor cells (such as a lysosomal protease) could be used as an efficient drug delivery system. The polymer should selectively accumulate at the tumor site due to the EPR effect and will then be activated by a lysosomal protease upon endocytosis into cancerous cells. The slow release of the drug molecule is expected to occur inside the cell and, therefore, nonspecific drug release should not occur.

In conclusion, we have developed a novel self-immolative polymer based on a polyurethane backbone. This comb-polymer underwent complete disassembly to release multiple reporter groups upon a single activation event at its head. The polymer was prepared by simple polymerization of phenylcarbamate monomers, followed by capping of the polymer head with trigger. This synthetic technique allowed us to rapidly obtain a polymeric molecule with large number of reporter units. A water-soluble prototype of a self-immolative comb-polymer was activated under physiological conditions by the protease PGA. Use of drug molecules instead of the reporter units will generate a polymeric drug delivery system that can selectively release a high payload of drug upon stimulus by specific enzyme. We are currently developing self-immolative polymeric systems with faster release mechanism.

Acknowledgements

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